平成２３年度福井大学研究育成経費「若手研究者による今後の進展が期待できる研究」

Comparative Study of Raman micro spectroscopy and FTIR in Colorectal Tumor Model

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概　　要	FTIR spectroscopy and Raman micro-spectroscopy were used to differentiate between grade levels of non clinic samples, colorectal tumor models at 4 different grades; normal, grade 1, grade 2 and grade 3. Detailed investigation of the spectra in the fingerprint region (4000–500 cm^{-1}) revealed some distinct peaks and shoulders, most of which were assignable to wave numbers that shown to represent biochemical changing within the tissue. Differences in peak heights and peak ratios indicated differences in biochemical composition of cancer from different grade level. It was possible to distinguish between their grades. However, all collected colorectal tumor model at different peak was distinguishable using Raman micro-spectroscopy and FTIR clearly. The nucleus information (grouping in PCR) of H-E (hematoxylin-eosin) staining image is very nice correlation with P=O component. Antisym. P=O component (stretch. vib.) has higher correlation with antisym stretch. of CH_2 and C=O component at 5°C compared with sym. P=O component. (FTIR data). Sym. P=O component has higher correlation with antisym. stretch. of CH_2 and C=O component at -5°C compared with antisym. P=O component. (FTIR data). Raman data has the same results with FTIR data.
関連キーワード	FTIR, Raman Spectroscopy, colorectal tumor, Antisymmetric Phosphate

研究の背景および目的

Application of H-E staining in histopathology has had a play role in diagnosing the tissue. However, several researchers report that some biochemical content may be removed due to some chemical that are used during the H-E staining process. Therefore, we proposed the progressed a FTIR Microscope and Raman micro-spectroscopy for observation the fresh tissue. The different temperature of sample holder will be used for testing whether there will be temperature dependency or not during the measurement. We emphasized to antisymmetric and symmetric P=O stretch. vib. due to suggest an alteration of their content in the nucleic acids in the malignant tissues.[3]

Starting the last decade, FT-IR micro-spectroscopy has already increased in capabilities to visual a molecular component for unstained tissue.[1] IR spectroscopy is used to excite the vibrational motion of covalently bonded moieties by direct absorption of the photon.[2] Raman micro-spectroscopy is a non-invasive method by which information regarding molecules and crystallization on the surface of cryo-sectioned tissue can be obtained at room temperature.[4]

研究の内容および成果

Colorectal tumor model (CTM) were established by intra-peritoneally administrated of Azoxymethane to the Balb/c mouse. Samples were directly harvested after 12 weeks and continuing for frozen section. The first of serial transversal section (± 6 µm in the thickness size) was mounted onto a slide glass, and stained with hematoxylin and eosin; the second serial transversal section (± 8 µm) was for FT-IR 3000M observation through attaching the sectioned sample on the BaF_2 plate gently and directly kept them under -21°C. The third was for Raman micro-spectroscopy observation.

Result and Discussion

Figure 1A showing the grouping of H-E section and transmitted picture (1B) where consist of N (normal group), G1 (group 1), G2 (group 2) and G3 (group 3). Grouping was as non clinical diagnosing for decision of malignancy and based on nucleus phenomena, including the differences in size and color of cells. Therefore, the determined groups are as a temporary grouping.

Using PCR method, the peak intensity of antisymmetric and symmetric P=O stretch. vib. show some differences. The different temperature treatment make some distinct to both of phosphordiester (Fig. 2). Fig. 2A and 2D showing the 3 dimension of antisymmetric P=O stretch. vib. which is clearly similar with the high peak intensity of 2 dimension (Fig 2B & 2E).

Fig. 2C & 2F, showing the superimpose of FTIR observation for antisymmetric P=O stretch. vib. that detected as the intensity of reddish color at 5°C & -5°C. The application of Raman spectroscopy and FTIR demonstrated the similar result where both of devices detect that antisymmetric P=O stretch. vib. is more higher than symmetric P=O stretch vib. at temperature more than 5°C. In focusing to the mapping image, it looks that antisym. P=O stretch. vib. reflection is higher in reddish color compared with the mapping imaging of sym. P=O stretch. vib.. The implication of it that Antisym. P=O stretch. vib. have a higher accumulation within CTM.Table 1 describe both of the mean-peak Raman intensity and FTIR reveal the higher peak intensity of Antisymmetric P=O stretch. vib. in the normal group. While in the group-2, symmetric P=O strect. vib. depict oppositely.

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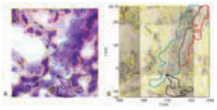

Fig 1 . Grouping based on H-E staining (A) transmit picture (B).

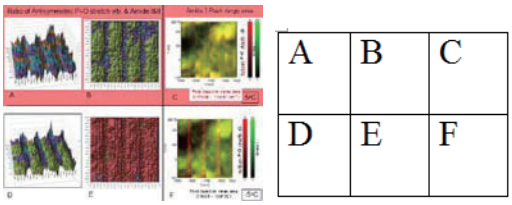

Fig.2. Demonstrating the reconstruction of 3 dimension (A & D) and 2 dimension (B & E), and the superimpose of FTIR observation (C & F).

A

Classification Cells group	Phosphor diester group: Antisymmetric P=O Stretch. Vib. (1223 cm⁻¹)	Phosphor diester group: Symmetric P=O Stretch. Vib. (1078 cm⁻¹)
Normal-group (normal cells)	0.023	0.026
G2-group (Adenoma)	0.017	0.047

B

Classification Cells group	Phosphor diester group: Antisymmetric P=O Stretch. Vib. (1223 cm⁻¹)	Phosphor diester group: Symmetric P=O Stretch. Vib. (1078 cm⁻¹)
Normal-group (normal cells)	0.26	0.28
G2-group (Adenoma)	0.12	0.18

Table 1. The comparative of the mean-peak of Raman intensity (A) and FTIR (B) for phosphordiester group within CTM.

本助成による主な発表論文等、特記事項および競争的資金・研究助成への申請・獲得状況

「主な発表論文等」

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「競争的資金・研究助成への申請・獲得状況」

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